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Abstract: Background/Aims. Recently, peripheral blood mononuclear cell transcriptome analysis has identified genes that are upregulated in relapsing minimal-change nephrotic syndrome (MCNS). In order to investigate protein expression in peripheral blood mononuclear cells (PBMC) from relapsing MCNS patients, we performed proteomic comparisons of PBMC from patients with MCNS in relapse and controls. Methods. PBMC from a total of 20 patients were analysed. PBMC were taken from five patients with relapsing MCNS, four in remission, five patients with other glomerular diseases and six controls. Two dimensional electrophoresis was performed and proteome patterns were compared. Results. Automatic heuristic clustering analysis allowed us to pool correctly the gels from the MCNS patients in the relapse and in the control groups. Using hierarchical population matching, nine spots were found to be increased in PBMC from MCNS patients in relapse. Four spots were identified by mass spectrometry. Three of the four proteins identified (l-plastin, -tropomyosin and annexin III) were cytoskeletal-associated proteins. Using western blot and immunochemistry, l-plastin and -tropomyosin 3 concentrations were found to be enhanced in PBMC from MCNS patients in relapse. Conclusions. These data indicate that a specific proteomic profile characterizes PBMC from MCNS patients in relapse. Proteins involved in PBMC cytoskeletal rearrangement are increased in relapsing MCNS. We hypothesize that T-cell cytoskeletal rearrangement may play a role in the pathogenesis of MCNS by altering the expression of cell surface receptors and by modifying the interaction of these cells with glomerular cells

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Proteomic analysis of mononuclear cells of patients with minimal-change nephrotic syndrome of childhood

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Abstract

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Keywords: cytoskeleton; L-plastin; minimal-change nephrotic syndrome; mononuclear cells

Introduction

Idiopathic nephrotic syndrome is the most frequent glomerular disease in childhood. It is characterized by massive proteinuria, and histopathological changes are restricted to effacement of podocyte foot processes without significant glomerular immune deposits [minimal-change nephrotic syndrome (MCNS)]. Several clinical and experimental studies underscore the implication of T-cells in the pathogenesis of MCNS. For example, immunogenic stimuli such as viral infections, immunizations and allergens have been shown to trigger nephrotic relapse. In addition, remissions can be induced by steroids and calcineurin inhibitors (ciclosporin and tacrolimus). The efficacy of thymodependent cell cytotoxic agents such as cyclophosphamide and chlorambucil observed during MCNS relapses represents further evidence implicating an immune pathogenesis [1]. Thus, MCNS is considered to be associated with T-cell dysfunction; however, the molecular link between the immune system and the kidney is still unknown.

Recently, several groups attempted to identify the T-cell genes that were involved in MCNS using a substractive cDNA library [2] and serial analysis of gene expression (SAGE) [3,4]. These studies revealed several differences in the transcriptome of peripheral blood mononuclear cells (PBMC) in relapsing MCNS.

In order to investigate the differences in protein expression in PBMC from relapsing MCNS patients, we performed proteomic comparisons of PBMC from patients with MCNS in relapse and remission, patients with other glomerulopathies and non-proteinuric patients. Spots selectively upregulated in MCNS in relapse were further identified by mass spectrometry.

Materials and methods

Patients

Samples were drawn from four groups of patients: five patients with MCNS in relapse, four in remission, five patients with other glomerular diseases and six non-proteinuric controls. For each patient, the clinical history and laboratory

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data were obtained (Table 1). The criteria of the International Study of Kidney Disease in Children were used for the diagnosis and management of MCNS [5].

PBMC collection

Blood samples were obtained from patients cared for at the Children's Hospital of Geneva and the Kinderspital of Zurich. The study was approved by the local ethical committees. For each patient, 10 ml of blood was collected in an Acid-Citric-Dextrose tube (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA) and PBMC were separated by gradient centrifugation (Ficoll-Paque, Amersham Biosciences, Freiburg, Germany) and frozen in autologous plasma with DMSO in nitrogen. In order to compare our results with those obtained from non-specific stimulation of PBMC, a group of stimulated PBMC from controls was included, and stimulation was achieved using phytohemagglutinin (PHA-P) (Sigma, St Louis, MO, USA) as follows: cells were resuspended in RPMI (Invitrogen System, CA, USA) and 20% autologous plasma with PHA 10 μg/ml at a density of 2×10^6 cells/ml. Cells were incubated overnight at 37°C/5% CO_2. After gentle centrifugation, supernatant was discarded and cell pellet frozen at −80°C.

T-cell enrichment of PBMC

T-cells were isolated from PBMC using immunomagnetic negative cell selection (StemSep, StemCell Technologies Inc., British Columbia, Canada). PBMC were suspended in a mixture of antibodies that included anti-CD14, CD16, CD19, CD56 and anti-glycophorin A (StemSep ANTIBODY COCKTAIL, StemCell Technologies Inc.) and incubated with a magnetic colloid (StemSep MAGNETIC COLLOID, StemCell Technologies Inc.). The resulting cell suspension was loaded on a column and an enriched suspension of unlabelled cells was collected. Cell purity of T-cell enriched PBMC and T-cell and B-cell repartition of PBMC obtained from controls, MCNS patients in relapse and remission were tested by FACS using CD3, CD14 and CD19 antibodies (DAKO, Denmark).

2D PAGE

Urea was from Merck (Darmstadt, Germany), Thiourea was from Fluka (Buchs, Switzerland) and Zwittergen from Calbiochem (CA, USA). CHAPS and Iodoacetamide were from Sigma and DTT from Amersham Biosciences.

PBMC isolated from each patient's sample were resolved in a single 2D gel. Gels were performed according to Görg with some modifications [6]: cellular proteins (125 μg of proteins from 1×10^6 cells) were solubilized using a lysis buffer containing 7 M urea, 2 M thiourea, 2% CHAPS, 2% Zwittergen and 65 mM dithiothreitol [7]. In-gel rehydration was performed overnight at room temperature. Isoelectrofocusing was performed on the Multiphor II system (Amersham Biosciences) under paraffin oil using a nonlinear IPGs pH range 3–10, 18 cm (Immobiline DryStrips 3–10 NL, Amersham Biosciences). The voltage was progressively increased to a maximum of 3500 V and for a total of 80 kVh.

Before the second dimension, strips were equilibrated in a solution containing 6 M urea, 50 mM Tris–HCl, 30% glycerol, 2% SDS, 1% DTT for 20 min, and a second time in the same solution containing traces of bromophenol blue and 4.8% iodoacetamide. The second dimension was performed using precast polyacrylamide gels 12–14% gradient (5–120 kDa) (ExcelGel XL SDS 12–14, Amersham Biosciences). Migration was started at 150 V, 20 mA for 1 h, and 800 V, 35 mA for 3 h, at 18°C. Analytic gels were silver stained (Silver staining kit, Amersham Biosciences) and dried overnight. For pattern evaluation, gel images were captured using CCD Fujilas 1000 Plus (Fujifilm Medical Systems, USA).

To demonstrate differences in protein expression in the PBMC from relapsing MCNS patients, we used two methods, heuristic clustering and hierarchical population matching.

Heuristic clustering. Heuristic clustering was performed for the automatic classification of a first set of gels using a previously described algorithm [8]. 2D gels were obtained from four different samples of PBMC of MCNS patients in relapse and from three samples of control PBMC. The algorithm provides the possibility of blindly classifying similar gels into a determined number of classes (in this case, relapse and controls).

Hierarchical population matching. For each gel, spot detection, landmark editing and pattern matching were performed using the Melanie software version 3.0 (Genebio, Geneva, Switzerland). Gels were compared using a hierarchical population matching. For each condition studied, gels were matched and the group of spots merged into an average gel. All average gels were added to a mastergel that served as a reference gel. All gels, independently of their clinical classification, were then matched with the reference gel. For spot quantification, a relative spot volume determination (volume of a spot/volume of all spots in the gel) was used as it is a normalized value that is relatively independent of variations between gels due to experimental design. Gels were then matched and histograms for groups based on normalized % volume of the spots were recorded, using the central tendency as the mean value and the mean square deviation as the dispersion value. The spots displaying a change in their expression between classes greater than the twofold factor (determined from the mean) were considered for protein identification.

In order to proceed to the identification of the selected spots, preparative gels were performed with a higher protein load (0.6–1 mg of proteins from 5–8 $\times 10^6$ cells). For protein detection, gels were fixed for 30 min in 10% methanol and 7% acetic acid and stained overnight using SYPRO Ruby (Molecular Probes, USA) [9]. Gels were digitalized at 470 nm and spots of 1.4 mm diameter picked using a 300 nm light. Gel plugs were then destained and incubated in 75% acetonitrile for 20 min. For trypsinisation, gel plugs were incubated with trypsin for 1 h at 37°C and peptides extracted with an acetonitrile-TFA solution. Identification was performed using a 4700 Proteomics Analyzer mass spectrometer (Applied Biosystems, Foster City, CA, USA) mass spectrometer and an

Table 1. Characteristics of patients used for 2D gels comparison. PBMC from two patients (3 and 4) were analysed both in remission and in relapse

Patients	Sex	Age (years)	Clinical diagnosis	Years since diagnosis	Renal biopsy	Steroid therapy	Other treatment	PU (g/l)	Urinary protein/creatinine ratio (g/mol)	Plasma protein (g/l)	Plasma albumin (g/l)	Creatinine clearance (ml/min)
1	F	11	MCD remission	3	–	N	N	0.28	43.8	69	44	102
2	F	9	MCD remission	1.5	–	N	N	0.08	13.7	72	45	90
3	F	10.5	MCD remission	9	MCD	Prednisone 40 mg EOD	Ciclosporine 200 mg Qday	0.11	12.2	67	37	90
4	M	4	MCD remission	1	MCD	Prednisone 10 mg EOD	Ciclosporine 140 mg Qday	0.03	6.0	61	31	113
5	M	7	MCD relapse	1	–	N	N	10	2400.0	51	30	108
6	M	9	MCD relapse	5.5	–	Prednisone 30 mg EOD	Levamisole 50 mg EOD	5.12	1163.6	44	17	178
3	F	14	MCD relapse	7	–	Prednisone 5 mg EOD	N	4.16	343.8	46	18	142
4	F	10	MCD relapse	8	–	Prednisone 60 mg EOD	Ciclosporine 200 mg Qday	30	1435.4	48	11	121
7	M	3	MCD relapse	0.5	–	Prednisone 30 mg EOD	N	2.76	563.3	54	30	189
8	F	12.5	MPGN type 1	0.2	MPGN type 1	N	N	3.98	995.0	37	14	67
9	M	6.5	IgA	5	IgA	N	Enalapril 10 mg Qday	2.25	239.0	63	NA	70
10	F	10.5	IgA	0.1	IgA	N	N	1.06	342.0	62	33	133
11	F	15	IgA	1.5	IgA	N	Enalapril 10 mg Qday	1.09	129.8	68	40	87
12	M	17	IgA	3	IgA	N	Enalapril 15 mg Qday	NA	33.0	68	40	79

MCD: minimal-change disease; IgA: IgA nephropathy; MPGN: membranoproliferative glomerulonephritis; N: no treatment; EOD: every other day; Qday: once a day; PU: proteinuria; NA: non-available.
Creatinine clearances were calculated using the Schwartz formula.

α-cyano-4-hydroxy-cinnamic acid matrix (Sigma). The identified peptides were matched against the Mascot database for protein identification.

Western blots

The mouse anti-human L-plastin antibody (clone LPL4A.1) (NeoMarkers, CA, USA) was used at 1/2500 in a blocking buffer (5% Nonfat Milk in Tween 20 Tris buffered saline). Total PBMC extracts (2×10^6 cells) were solubilized in a lysis buffer with 0.1% sodium dodecyl sulfate (SDS), 1% NP40, 1% Na-deoxycholate and protease inhibitor Complete (Roche Applied Science, IN, USA). Cell lysates were resolved by 10% SDS gel electrophoresis [10] and transferred to the polyvinyl difluoride (PVDF) membrane (Hybond-P, Amersham Biosciences) by electroblotting using semi-dry blotting and a discontinuous buffer system. Immunoblotting was performed as described [11] and detection was performed using enhanced chemoluminescence (ECL + kit, Amersham Biosciences). Membranes were exposed for 5–20 s to Hyperfilm ECL (Amersham Biosciences) in an X-ray film cassette. Variations among samples were assessed by incubation with the anti-GAPDH antibody (1/2500). After revelation, films were scanned with an image scanner (Amersham Biosciences) and quantified with the Image Quant TL software (Amersham Biosciences).

Immunohistochemistry

The mouse anti-human L-plastin antibody was purchased from Abcam (Cambridge, UK), mouse anti-human α-tropomyosin 3 antibody (clone CG1) from NeoMarkers and rabbit anti-human annexin III from Genex Bioscience Inc. (CA, USA).

PBMC were cytospun at 10^5 cells/slide, fixed with paraformaldehyde 4% and permeabilized with 0.3% Triton X-100 for 5 min at room temperature. Cells were incubated in the blocking solution (2% BSA in PBS) for 30 min at room temperature, and incubated overnight at 4°C with the antibody (1/100 for L-plastin, 1/1000 for α-tropomyosin 3). Slides were washed three times with PBS, and then incubated with the goat anti-mouse FITC labelled antibody (1/200 in the blocking solution).

Images were obtained using an Axiovert 200 M microscope (Zeiss, Germany) and processed with the Adobe Photoshop 5.5 software. All images were analysed according to a modification of the method of Chen *et al*. [12]. For each protein, a total of 70 cells were individually photographed at 100× magnification and scored by two investigators who were blinded to the identity of the samples. The samples were then scored from 0 (no signal) to 3 (highest expression) for L-plastin, α-tropomyosin 3 and annexin III. Each blinded experiment was carried out on three independent occasions.

Statistics

Results are given in terms of mean ± standard deviation. The differences in expression levels between relapsing MCNS and control conditions and patients with other glomerular diseases and control conditions for a given protein were assessed using the two-sample Mann–Whitney test. The relationship between spot relative volume and the patient's steroid dosage was examined using linear regression analysis. Differences were considered statistically significant for $P < 0.05$.

Results

Comparison of T-cell enriched PBMC and PBMC

The 2D gel proteomes of T-cell enriched PBMC and unfractionated PBMC were compared using the Melanie Software. For T-cells, an average of 1248 ± 166 spots (mean ± SD) and for PBMC, an average of 1254 ± 29 spots (mean ± SD) were detected per gel. The matching between T-cell gels and PBMC did not reveal any major pattern differences as spots present in T-cell gels were accurately represented in PBMC gels. Due to limited amount of samples, all further comparisons were carried out on PBMC. The percentage of T-cells in PBMC obtained from controls was 70.0 ± 6.5%, 72.1 ± 9.1% in MCNS patients in relapse and 77.7 ± 3.0% in MCNS in remission ($n = 5$; $P = 0.437$). The percentage of B-cells was 16.0 ± 1.9%, 14.9 ± 2.5% and 10.4 ± 1.0%, respectively ($n = 5$, $P = 0.126$).

Protein alterations in PBMC from MCNS patients

Heuristic clustering analysis. The heuristic clustering analysis after the image processing of seven 2D gels (three gels of PBMC from control patients and four gels of PBMC from patients with MCNS in relapse) correctly classified the gels into the two groups.

2D gel comparison. In order to determine which proteins were upregulated in PBMC of patients with MCNS in relapse, five groups of samples were processed: a group of five samples of PBMC from patients with MCNS in relapse, a group of four samples from patients with MCNS in remission, a group of five samples of PBMC from patients with other glomerular diseases, a control group of six samples from non-proteinuric patients and a group of five samples of PHA-stimulated PBMC from control patients.

A gel with a pH range 3–10 was performed for each sample (total of 25 gels). A reproducible protein pattern was achieved and an average of 1180 ± 301 spots per gel was detected. After image acquisition, gels were analysed using the Melanie software. Prior to matching, landmark editing was performed and a synthetic gel was created for each group of samples. Each gel was matched to a mastergel in order to compare proteome patterns.

After gel analysis, nine spots with an increased expression in gels from patients with MCNS in relapse were selected for identification from which two could not be picked on the preparative gel and therefore could not be further analysed. Of the remaining seven spots analysed with the mass spectrometer, three had a very weak mass spectrometry signal and could not be further analysed and four spots were identified with a good database matching. The four proteins identified were L-plastin,

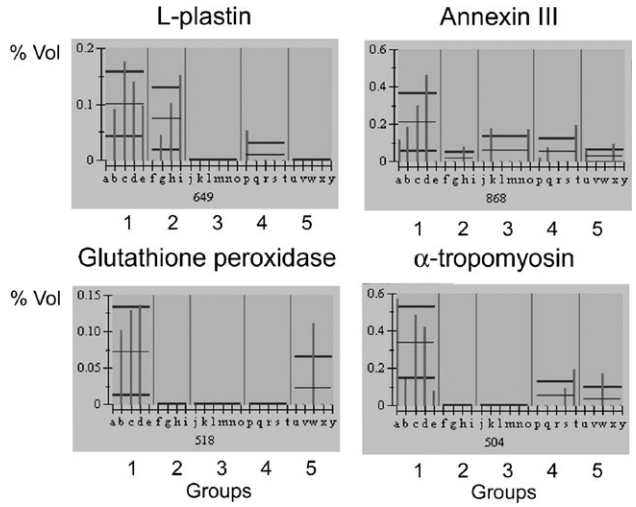

Fig. 1. Histogram showing the relative volume of the spots analysed by mass spectrometry: for spot quantification, a relative spot volume determination (volume of a spot/volume of all spots in the gel) was used as it is a normalized value that is relatively independent of variations between gels due to experimental design. On the *x*-axis, each letter represents a single gel and each number represents a group (1: relapse, 2: remission, 3: control, 4: other glomerular diseases, 5: PHA stimulation). The *y*-axis represents the relative volume of the spot.

Table 2. Spots with an upregulated expression in PBMC from patients with relapsing MCNS

Spot no.	Protein name	Relapse versus control and remission	Other glomerular diseases versus control and remission
2	Annexin III	0.04	0.631
13	Glutathione peroxidase	0.009	1.000
15	α-Tropomyosin	0.01	0.254
6	L-plastin	0.05 (0.010[a])	0.699

[a]Compared to control patients only.
The differences in expression levels between MCNS patients in relapse and controls and in patients with other glomerular diseases and controls for a given protein were assessed by using the two-sample Mann–Whitney tests.

annexin III, α-tropomyosin 3 and glutathione peroxidase (Figure 1 and Table 2). The proteins characteristics are described in Table 3.

The relationship between spots' relative volume in the patient's gel and the patient's daily steroid exposure showed an r^2 at 0.17 ($P = 0.15$) for L-plastin, 0.13 ($P = 0.20$) for annexin III, 0.07 ($P = 0.36$) for α-tropomyosin 3 and 0.18 ($P = 0.13$) for glutathione peroxidase.

Western blots and immunocytochemistry of L*-plastin, α-tropomyosin 3 and annexin III.* Densitometric analysis of L-plastin western blots was performed in PBMC protein extracts from seven MCNS patients in relapse and eight controls. L-plastin concentration was 2010 ± 168 DU in controls and 2588 ± 193 DU in MCNS patients in relapse ($P < 0.05$). The L-plastin–GAPDH ratio increased from 0.9 ± 0.08 to 1.2 ± 0.09 ($P < 0.05$).

The expressions of L-plastin, α-tropomyosin 3 and annexin III were also examined immunocytochemically in PBMC from MCNS patients in relapse ($n = 3$) and from controls ($n = 3$). The expression of L-plastin and α-tropomyosin 3 was enhanced in PBMC from MCNS patients in relapse (Figure 2). Scores were 1.33 and 1.55 in controls and 2.19 and 2.56 in relapse ($P < 0.0001$). No changes were observed for annexin III.

Discussion

This study was designed to identify proteins specifically upregulated in mononuclear cells of MCNS patients in relapse using a proteomic approach. Comparisons of mononuclear cell proteomic maps from patients with MCNS in relapse, remission, other proteinuric conditions and controls were performed using heuristic clustering and hierarchical population matching. Heuristic clustering takes into account several small differences in spot patterns that may not be individually recognized by the hierarchical population. The ability of heuristic clustering to correctly classify the gels into two groups (MCNS patients in relapse and controls) demonstrates the existence of a specific pattern of spots in gels of PBMC from patients with MCNS in relapse.

Among the four upregulated spots in MCNS in relapse identified by hierarchical population matching and mass spectrometry, three were cytoskeletal-associated proteins: L-plastin, annexin III and α-tropomyosin.

The first protein, L-plastin, is a leukocyte-actin-binding protein and recent studies have implicated L-plastin phosphorylation in the regulation of β2 integrin function [13] whose activation and binding to ICAM-1 on the endothelial surface are part of the process of homing in leukocytes. Furthermore, phosphorylation of L-plastin was found to play a crucial role for the transport of activation-induced receptors to the surface of T-cells [14]. L-plastin's transcript was found to be upregulated using a cDNA library in PBMC from MCNS patients in relapse [2], and in our study, the upregulation of L-plastin was confirmed by western blot and immunochemistry. Therefore, the upregulation of L-plastin could be implicated in T-cell activation in MCNS in relapse.

The second protein, annexin III, is an abundant neutrophil cytosolic protein that was found to influence the function of membrane constituents by association and dissociation with the inner membrane in concert with calcium oscillations [15]. Furthermore, a 36 kDa annexin III variant has been identified in human monocytes and could play a specific role in these cells [16].

The third protein, α-tropomyosin, binds to actin filaments in muscle and non-muscle cells and is implicated in stabilizing cytoskeleton actin filaments.

The upregulation of these three cytoskeletal-associated proteins in MCNS in relapse might therefore emphasize a potential role of T-cell activation in the pathogenesis of MCNS.

Several authors had analysed mononuclear cells of MCNS patients using genomics: Sahali isolated 84 transcripts using a subtracted cDNA library screening. Among the 42 known transcripts, 18 were involved in the T-cell signalling cascade, including genes encoding cytoskeleton-associated proteins such as L-plastin, grancalcin and

Table 3. Spots with an upregulated expression in relapsing MCNS: mass spectrometry and Swiss-Prot database results with entry name, accession number, theoretical isoelectric point, molecular weight and major functions

Spot no.	Protein name	No. of peptide observed by PMF	MASCOT score	Sequence coverage (%)	Swiss-Prot accession no.	MW (kDa)	p*I*	Major functions
2	Annexin 3	4	69	13	P12429	36.4	5.6	Family of calcium binding proteins. Implicated in exocytose, formation/modulation of ion channels and membrane attachment of cytoskeletal elements
6	L-plastin	10	87	21	P13796	70.3	5.2	Leukocyte-actin-binding protein. Might be implicated in integrin activation and adhesion via its phosphorylation
13	Glutathione peroxidase	na	41	na	P07203	21.9	6.1	Regulation of phospholipid turnover and protection against oxydative stress injury
15	α-Tropomyosin isoform TM3	na	23	na	P06753	32.8	4.7	Binds to actin filaments in muscle and nonmuscle cells. Implicated in stabilizing cytoskeleton actin filaments

na: not available.

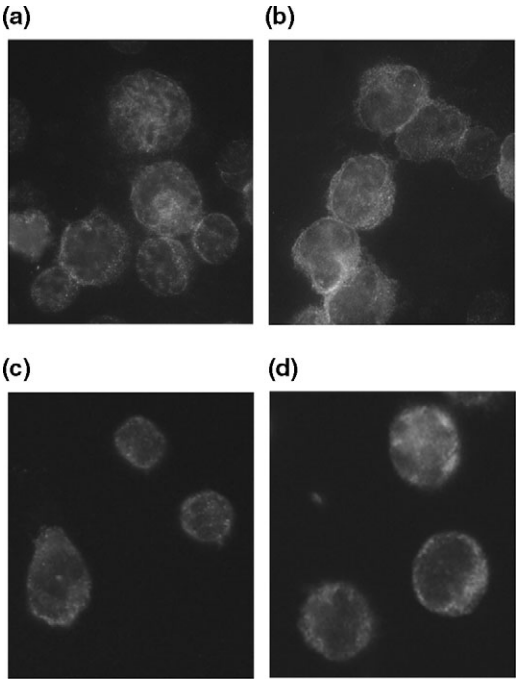

Fig. 2. The expression of L-plastin and α-tropomyosin observed by immunohistochemistry. The expression of L-plastin in PBMC from controls (**a**) and MCNS patients in relapse (**b**). The expression of α-tropomyosin in PBMC from controls (**c**) and MCNS patients in relapse (**d**).

Fyb/Slap [2]. Using SAGE methodology, Mansour found 179 tags over-represented in the relapse library including 21 transcripts coding for T-cell surface markers, numerous transcripts encoding proteins involved in protein metabolism, energy supply and signal transduction [3]. Using the same SAGE technology, Okuyama found 15 genes upregulated in relapsing MCNS [4]. These studies were conducted in only isolated individuals and in our experiments we used a hierarchical population matching comparing several individuals in each group to minimize the individual differences not related to the disease itself. Studies have been published recently that compare both genes and proteins that are differentially expressed in two biological conditions [17]. The conclusion drawn by the authors is that there is no or only a moderate correlation between differential RNA and protein expression.

In our study, only a few spots could be identified as upregulated in MCNS in relapse and this might be due to methodological constraints: only spots with a mean twofold increase in relative volume were selected for identification and the proteomic technique itself is poorly efficient in separating basic proteins, proteins with low molecular weight and membrane proteins. Further, proteomic analysis using the DIGE technology or isolation of membrane proteins could help us identify more proteins even though a higher amount of material would be needed in order to proceed with these experiments.

We were also able to address the question of the specificity of the changes in protein expression. The analysis of multiple control conditions (remission, other glomerular diseases and nonproteinuric patients) was conducted in order to minimize differences in protein expression secondary to treatment or other factors not related to the MCNS disease itself. As proteins identified in MCNS patients in relapse were implicated in cell activation, we obtained a nonspecific stimulation of PBMC from control patients using a mitogen agent, PHA. None of the identified spots were upregulated in the PHA-stimulated PBMC thus indicating that the observed modifications were unlikely secondary to non-specific stimulation of PBMC.

In our study population, four of the five patients with MCNS in relapse and two of the four patients with MCNS in remission were treated with prednisone every other day, and we can address the question of the influence of the steroid therapy on protein expression. The

immunosuppressive effect of glucocorticoids on peripheral T-cells has been widely described in the literature [18] and in proteomic studies, a recent paper from Billing [19] analysed the cortisol-mediated stress response in a human monocytic cell line THP-1 by incubating them with cortisol for 48 h. Proteomic maps were then compared using the DIGE technology. Differentially expressed proteins (>1.5-fold) were selected for identification. Upregulation of 28 proteins was found with the highest upregulation for FKBP51. Other identified proteins were involved in cytoskeleton, chaperoning, metabolism, transcription and immune response. L-plastin and isoforms were found to be upregulated 1.5- to 2.2-fold. In our study, patients in both groups (relapse and remission) were treated with steroids and amongst the four patients on steroid with MCNS in relapse, only one was on a high dose (60 mg) and one was on a very low maintenance dose (5 mg). No correlation was found between daily steroid exposure and expression of L-plastin, annexin III, α-tropomyosin and glutathione peroxidase, and therefore, steroid treatment did not seem to have affected our results significantly.

In PBMC from relapsing MCNS patients, genomic studies have shown an upregulation of genes implicated in T-lymphocyte cytoskeletal modifications leading to cell signalling [2]. By using proteomics, our study showed upregulation of cytoskeletal-associated proteins involved in T-cell activation in MCNS in relapse thus providing further evidence for the link between the immune system and the renal cells in MCNS. We could therefore hypothesize that T-cell cytoskeletal rearrangement plays a role in the pathogenesis of MCNS by altering the expression of cell surface receptors and by modifying the interaction of these cells with glomerular cells.

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Conflict of interest statement. None declared. The results presented in this paper have not been published previously in whole or part, except in abstract format.

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